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Orientation of the Cleavage Site of the Herpes Simplex Virus Glycoprotein G-2

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During the synthesis of glycoprotein G-2 (gG-2) of herpes simplex virus type 2, the 104,000-Da gG-2 precursor (104K precursor) is cleaved to generate the 72K and the 31K intermediates. The 72K product is processed to generate the mature gG-2 (molecular mass, 108,000 Da), while the 31K product is additionally processed and secreted into the extracellular medium as the 34K component (H. K. Su, R. Eberle, and R. J. Courtney, J. Virol. 61:1735–1737, 1987). In this study, the orientations of the 31K and 72K products on the 104K precursor were determined by using two antipeptide sera produced in rabbits and a monoclonal antibody, 13αC6, directed against gG-2. The sera prepared against synthetic peptides corresponding to the terminal amino acid residues 67 to 78 and an internal peptide at amino acids 247 to 260 of gG-2 recognized the 104K precursor and the 31K cleavage product but not the 72K intermediate. In contrast, 13αC6 detected the 72K cleavage product and the uncleaved precursor but not the 31K cleavage component. The epitope recognized by 13αC6 was mapped within amino acids 486 to 566. These results suggest that the 31K cleavage product is derived from the amino-terminal portion of the 104K precursor molecule and that the 72K intermediate is derived from the carboxyl terminus. In support of our model described above for the synthesis of gG-2, antibodies recognizing either of the cleavage products reacted with the uncleaved precursor but not with the other cleavage product. By using partial endo-β-*N*-acetylglucosaminidase H analysis, two N-linked glycosylation sites were found on each of the cleavage products. The distribution of the N-linked glycosylation sites and the reactivities of the antipeptide sera allowed the cleavage region on the precursor to be mapped to within amino acids 260 to 437.

At least eight virus-specific glycoproteins in herpes simplex virus (HSV)-infected cells have been characterized, and they are designated gB, gC, gD, gE, gG, gH, gI, and gJ (see review by Roizman and Sears [23]). Recently, two additional glycoproteins have been identified and are referred to as gK and gL (14). Of these 10 glycoproteins, only glycoprotein G-2 (gG-2) of HSV type 2 (HSV-2) has been shown to undergo a unique cleavage event during its synthesis (5, 25). The cleavage of the gG-2 precursor results in the generation of the virion and the cell-associated mature form of gG-2 with an apparent molecular mass of 108,000 Da (108K glycoprotein), along with a 34K component that is secreted from the cell into the extracellular medium (25). In addition, it has been shown that this cleavage event can occur in the absence of an HSV-2 infection in gG-2-transformed cells (24). This cleavage-processing event has not been observed in the synthesis of any other HSV glycoproteins, including that of gG-1 of HSV-1 (1, 10, 16, 21).

The gene encoding gG-2 has been located within the US4 open reading frame which encodes a predicted protein product of 699 amino acids (17), and in vitro translation of the gG-2 mRNA results in the synthesis of a nonglycosylated 100K protein. This 100K nonglycosylated precursor is not detectable within infected cells and appears to be cotranslationally glycosylated by the addition of high-mannose sugars to a 104K intermediate (29). Subsequent processing of the 104K intermediate results in the cleavage event mentioned above, the modification of the high-mannose carbohydrate chains, and the apparent addition of O-linked sugars (5, 8, 15, 18, 22). Although four potential N-linked glycosylation sites can be deduced from the predicted protein sequence, the precise utilization and distribution of these sites on the cleavage products are unknown. The arrangement of the cleavage products and the location of the cleavage site on the gG-2 precursor also remain to be determined. In this study, the orientations of the two cleavage products of the gG-2 precursor were mapped by using site-specific antibodies directed against defined regions of the total gG-2 amino acid sequence. Additionally, the locations of the potential N-linked glycosylation sites on the cleavage products were determined by partial digestion with the endoglycosidase endo-β-*N*-acetylglucosaminidase H (endo H). The pattern of reactivity of the antisera with the cleavage products and the distribution of the N-linked glycosylation sites on the peptides allow a probable region for the proteolytic cleavage of the gG-2 precursor to be defined.

In order to establish the precise precursor-product relationships of the intermediates involved in the synthesis of gG-2, the processing of the 104K precursor protein into its cleavage products was investigated by pulse-chase isotopic labeling of gG-2. Parallel cultures of HSV-2-infected cells grown in the absence of inhibitors were labeled for 10 min with 200 μCi of [^{35}S]methionine per ml and chased for various times in the presence of excess cold methionine. gG-specific glycoproteins present in the cell lysates and extracellular medium were immunoprecipitated with mono-

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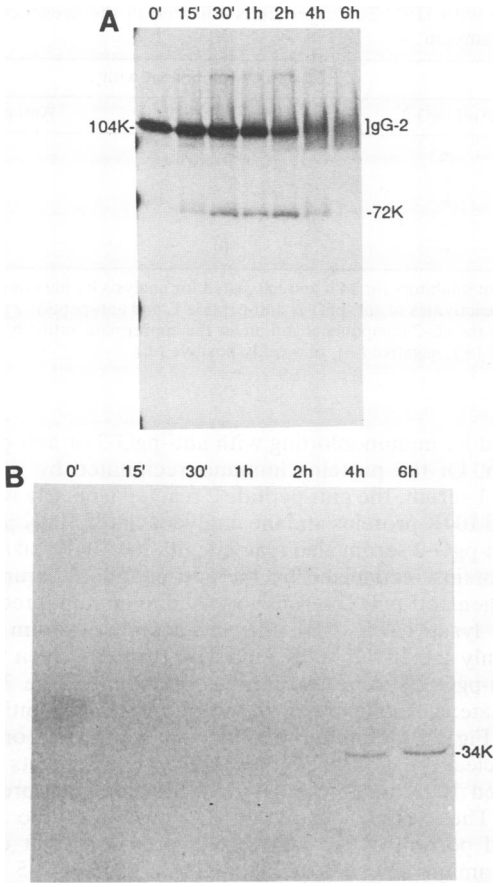

FIG. 1. Pulse-chase kinetics of synthesis of gG-2. HSV-2-infected cells were pulsed for 10 min at 12 h postinfection with 200 μCi of [^{35}S]methionine per ml and chased in excess cold methionine for the times indicated above each lane. At the indicated times, cell lysate (A) or medium (B) samples were harvested and immunoprecipitated with either monoclonal anti-gG-2 antibody (A) or polyclonal anti-pgG-2 serum (B). The immunoprecipitated proteins were analyzed by SDS-PAGE, and the proteins were visualized by autoradiography. The positions of the bands corresponding to the 104K precursor, the diffuse band of the 108K mature gG-2, and the 72K and 34K components are indicated.

clonal anti-gG-2 antibody 13αC6, which is reactive with the mature gG-2 (4) (Fig. 1A), or polyclonal anti-pgG-2 serum directed against the 104K precursor protein (Fig. 1B) and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), as previously described (19). The primary component detected within the infected cells during the 10-min pulse is the 104K precursor. Following the 10-min pulse, the intensity of the 104K precursor band diminishes with increasing times of chase. In concert with this reduction in the level of the 104K precursor, there is the transient appearance of the 72K band. With longer times, the amount of the 72K band is also seen to decrease, and this decrease is accompanied by the emergence of the diffuse gG-2 band (108K band). By 6 h of chase, only the mature gG-2 is detectable in the infected cells. In the extracellular medium, the 34K secreted component is detected after 2 h of chase and increasing amounts are observed with longer incubations (Fig. 1B). These results suggest that the 104K precursor is processed first into the 72K component, which is further modified into the mature (108K) gG-2. At the same time, this processing of the 104K component is accompanied by the secretion of the 34K component from the cell.

To determine the orientations of the 72K and 31K intermediates on the 104K precursor, antibodies against two synthetic peptides located within the amino-terminal portion of the gG-2 precursor were generated (Fig. 2). These synthetic peptides, designated peptides 1 and 2, correspond to amino acid residues 65 to 78 and 247 to 260, respectively, of the gG-2 precursor protein as deduced from the DNA sequence (17). The residues were selected on the basis of the method of prediction of antigenicity by Hopp and Woods (13). Peptides 1 and 2 (15 μmol each) were each conjugated to 20 mg of hemocyanin with glutaraldehyde (12). The conjugated products (5 mg) were emulsified in Freunds' incomplete adjuvant, and this solution was used to immunize rabbits to generate anti-peptide 1 and 2 sera (9).

In the first experiment, the reactivities of the two antipeptide sera were compared with that of the anti-pgG-2 antiserum by immunoblotting. Parallel cultures of mock-infected or HSV-2-infected cells grown in the presence or absence of monensin or tunicamycin were harvested at different times postinfection, solubilized, and analyzed by SDS-PAGE followed by immunoblotting (6, 28). The immunoblots were reacted with anti-peptide 1, anti-peptide 2, or anti-pgG-2 serum. As summarized in Table 1, in cells cultured without inhibitors the anti-pgG-2 serum detected the specific synthesis of the 104K precursor and the 72K and 31–34K (the 31K or its processed and secreted 34K form) cleavage products but recognized the mature gG-2 only poorly (24). In the presence of monensin, which disrupts normal Golgi processing and transport functions (27), the synthesis of the mature gG-2 and the 34K secreted component is inhibited (25). The precursors that accumulate within the cells include the uncleaved 104K protein, as well as the 72K and 31K (the intracellular precursor of 34K) cleavage products. The anti-pgG-2 serum detected the specific synthesis of the 104K precursor and the 72K and 31K cleavage products. Anti-peptide 1 and anti-peptide 2 sera both reacted weakly with the 104K precursor but strongly with the 31K intermediate. When N-linked glycosylation was inhibited by the addition of tunicamycin (26), the gG-2 precursor remained unglycosylated and was detected by anti-pgG-2 serum as the 100K nonglycosylated precursor (29). Both antipeptide sera recognized the uncleaved 104K precursor in the absence of both inhibitors and the 100K nonglycosylated form synthesized in the presence of tunicamycin. More importantly, both sera

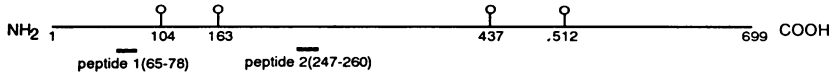

FIG. 2. Predicted gG-2 precursor. The linear structure of the gG-2 precursor from amino acid residues 1 to 699 is depicted. NH_2 denotes the amino terminus, and COOH denotes the carboxyl end of the protein. Synthetic peptides corresponding to amino acids 65 to 78 and 247 to 260 are indicated by two short bars. The four predicted N-linked glycosylation sites at amino acid positions 104, 163, 437, and 512 (17) are indicated by lollipops.

TABLE 1. Reactivities of anti-peptide 1, anti-peptide 2, and anti-pgG-2 sera with HSV-2-infected cells cultured in the presence or absence of monensin or tunicamycin

Component synthesized[a]:			Reaction of component with[b]:		
Without inhibitors	With monensin	With tunicamycin	Anti-pgG-2	Anti-peptide 1	Anti-peptide 2
108K			±	−	−
104K	104K		+	+	+
		100K	+	+	+
72K	72K		+	−	−
31–34K	31K		+	+	+

[a] HSV-2-infected cells were cultured in the presence of monensin or tunicamycin or without inhibitors for 14 h and harvested for analysis by immunoblotting.
[b] Immunoblots were reacted with anti-pgG-2, anti-peptide 1, or anti-peptide 2 serum. The reactivities of anti-pgG-2, anti-peptide 1, and anti-peptide 2 sera with each of the gG-2 proteins as determined by immunoblotting are indicated on the same line as the gG-2 components and under the appropriate antibody column. The reactivities of the different sera with the gG-2 intermediates are summarized as positive (+), negative (−), or weakly positive (±).

reacted well with only one of the cleavage products, the 31–34K component. Neither antipeptide serum detected the 72K cleavage product or its processed form, the mature gG-2.

In order to confirm that these antipeptide sera recognized the same proteins as the anti-pgG-2 serum, HSV-2-infected cells cultured in the presence or absence of monensin or tunicamycin were solubilized and immunoprecipitated (2) with either the anti-peptide 1 (Fig. 3A) or the anti-pgG-2 (Fig. 3B) serum. The immunoprecipitated proteins were analyzed by immunoblotting with anti-pgG-2 or anti-peptide 2 serum. Of the proteins immunoprecipitated by the anti-peptide 1 serum, the anti-peptide 2 reacted similarly with the 31K and 104K proteins and the unglycosylated 100K protein. The anti-pgG-2 serum also reacted with the 104K, 100K, and 31K proteins recognized by the anti-peptide 1 serum (Fig. 3A). When anti-pgG-2 serum was used to immunoprecipitate the cell lysates (Fig. 3B), the anti-peptide 2 serum recognized only the 104K, 100K, and 31K proteins, even though the anti-pgG-2 serum also immunoprecipitated the 72K intermediate, as shown by immunoblotting with the anti-pgG-2 serum (Fig. 3B). Both antipeptide sera recognized only one of the cleavage products, the 31K protein, and its unglycosylated 100K or glycosylated 104K uncleaved precursor forms. These results map the 31K protein to the amino-terminal portion of the 104K gG-2 precursor that encompasses amino acids 65 to 78 (peptide 1) and 245 to 260 (peptide 2). The assignment of the 31–34K component to the amino portion of the gG-2 precursor implies that the 72K intermediate is derived from the carboxyl terminus.

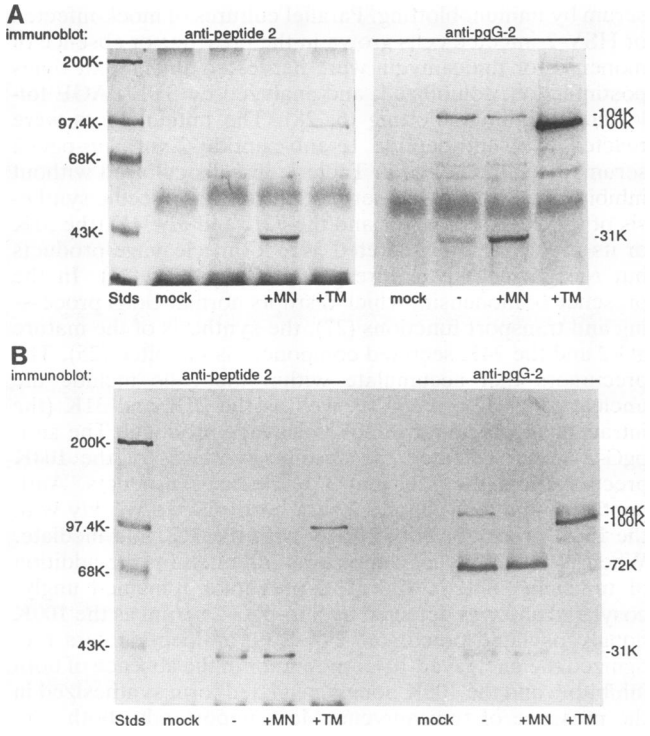

FIG. 3. Comparison of reactivities of anti-peptide 1, anti-peptide 2, and anti-pgG-2 sera. Mock-infected or HSV-2-infected cells grown in the absence (−) or presence (+) of 10^{-6} M monensin (MN) or tunicamycin (TM) (10 μg/ml) were harvested at 20 h postinfection and immunoprecipitated with either anti-peptide 1 (A) or anti-pgG-2 (B) serum. The immunoprecipitated proteins were analyzed by immunoblotting with either anti-pgG-2 or anti-peptide 2 serum as shown. Molecular weight standards (Stds) were included in the first lane of each panel, and the positions of the 104K, 100K, 72K, and 31K proteins are indicated.

Unlike the two antipeptide sera, 13αC6 has been shown to react with the 72K cleavage component, its mature 108K processing product, and the uncleaved 104K precursor but not with the 31–34K cleavage component. If the epitope recognized by 13αC6 maps within the carboxyl portion of the gG-2 precursor, then the positioning of the 72–108K component at the carboxyl end of the precursor molecule would be confirmed.

A series of carboxyl-terminal, truncated gG-2 derivatives were constructed by inserting chain termination linkers at various restriction enzyme sites within the gG-2 gene. Figure 4A schematically illustrates the various sites within the gG-2 protein at which a linker was inserted, and the numbers below the line indicate the number of gG-2 amino acids present within the various truncated proteins. The linker insertion mutant genes were subcloned into the mammalian cell expression vector pCDM8 (3) and transfected into COS-1 cells (11). Two days after transfection, the cells were incubated with [^{35}S]methionine for 2 h and harvested. The labeled proteins from the harvested cells and cell media were immunoprecipitated with 13αC6 (a monoclonal anti-gG-2 serum [4]) or the polyclonal anti-pgG-2 serum raised against the 104K gG-2 precursor. In COS-1 cells expressing the normal gG-2 protein, both the 104K precursor and the 34K product are precipitated by anti-pgG-2 (data not shown). When the polyclonal anti-pgG-2 antibody is used, a number of proteins are precipitated from cells expressing each of the truncated versions of gG-2 (data not shown). The monoclonal anti-gG-2 serum, 13αC6, precipitated truncated gG-2

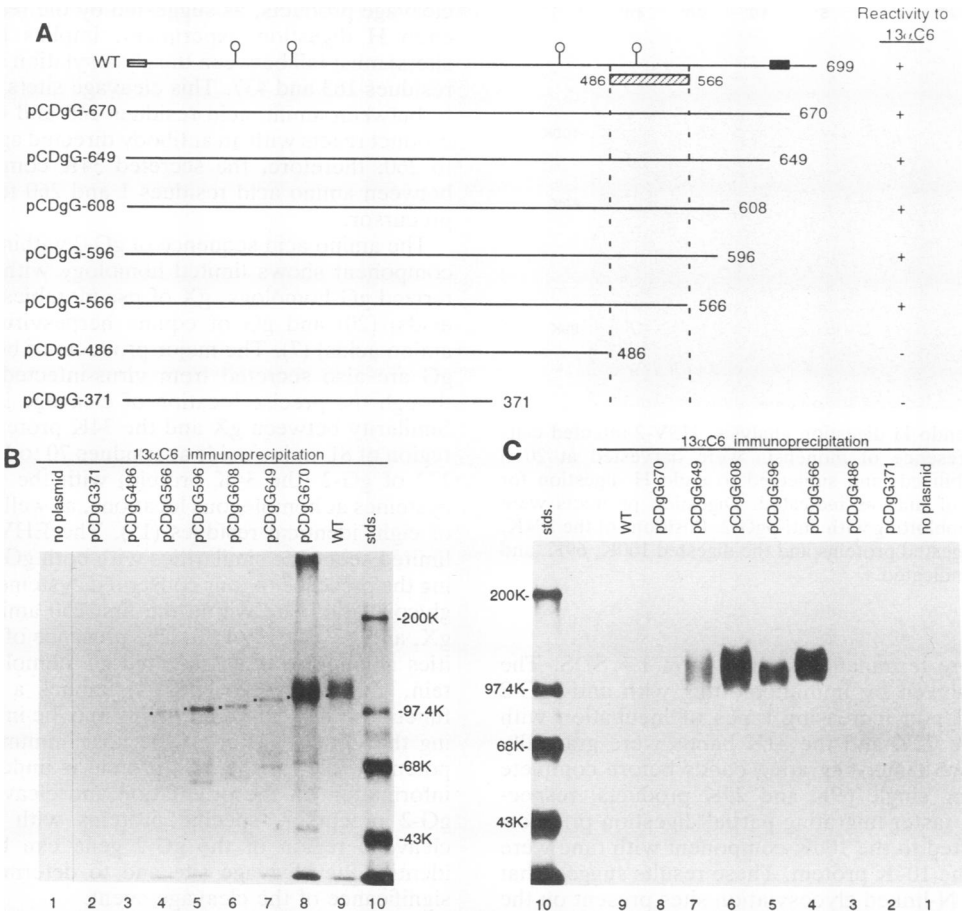

FIG. 4. Epitope mapping of 13αC6 monoclonal antibody. (A) A series of chain termination linker insertion gG-2 mutants was constructed to define the epitope recognized by 13αC6. Multiple-reading-frame termination linkers were inserted at various sites within the WT gG-2 gene in order to generate a series of truncation mutants, shown by the solid lines. Chain termination linkers were inserted at restriction sites *Nco*I, *Nar*I, *Nar*I, *Bss*HII, *Eco*RI, *Xba*I, and *Sty*I at nucleotides on the gG-2 open reading frame to generate the corresponding constructs pCDgG-371, pCDgG-486, pCDgG-566, pCDgG-596, pCDgG-608, pCDgG-649, and pCDgG-670. Truncated proteins containing the predicted number of gG-2 amino acid residues are indicated by numbers at the ends of the appropriate lines. The general location of the putative signal sequence (open box), transmembrane domain (solid box), and potential asparagine-linked glycosylation sites (lollipops) on the WT gG-2 are also indicated. A summary of the reactivity of 13αC6 with each of the truncated proteins is indicated. The epitope mapped in this study is depicted by the striped bar corresponding to amino acids 486 to 566. (B and C) COS-1 cells were transfected with different chain-terminating mutants of gG-2 with the indicated plasmids and labeled at 2 days posttransfection with 100 μCi of [^{35}S]methionine per ml for 2 h before harvest. The cell samples (B) or extracellular medium samples (C) were immunoprecipitated with 13αC6, analyzed by SDS-PAGE, and visualized by fluorography. Lanes 9, pCDgG699 (WT); lanes 10, molecular weight standards (stds.). The presumed uncleaved precursor of each mutant is denoted by a dot.

molecules containing the first 566 amino acids of gG-2 but not those which were 371 or 486 amino acids long (Fig. 4B). In cells transfected by plasmids pCDgG566 to pCDgG670 and the wild type (WT), 13αC6 precipitated major bands of increasing molecular weights corresponding to the presumed uncleaved precursors (Fig. 4B, dots) and one of the cleavage products (the truncated 72K intermediate). Except for pCDgG670 and the WT, no mature form of gG-2 (seen as a diffuse band in Fig. 4B, lanes 8 and 9) could be detected in these cells. Mature forms of truncated gG-2 from cells transfected with pCDgG566 to pCDgG649 but not from cells transfected with pCDgG670 or the WT were detectable in the extracellular medium (Fig. 4C), suggesting a loss of the membrane anchor sequence in the truncation mutant proteins consisting of 649 or fewer amino acids. The specificity indicates that the epitope recognized by 13αC6 lies between amino acids 486 and 566, and the reaction of the 13αC6 antibody with the 72K cleavage product or the mature 108K gG-2 (72–108K) confirms the location of the 72–108K protein to the carboxyl region of the gG-2 precursor.

From the DNA sequence of the gG-2 gene, four potential N-linked glycosylation sites (Asn-X-Ser/Thr) on the 104K gG-2 precursor are predicted. Whether all of these potential sites are glycosylated is unclear. In order to ascertain the number of these N-linked glycosylation sites that may be present on each of the cleavage products, the gG-2 precursor and its cleavage products were subjected to partial endo H digestion since both the 104K precursor and its cleavage products are sensitive to endo H digestion (29). HSV-2-infected cells cultured in the presence of monensin were solubilized and incubated with endo H (30) for various times. At the indicated times (Fig. 5), samples were digested, and

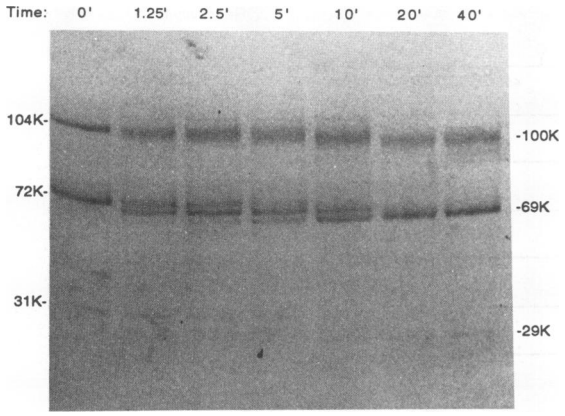

FIG. 5. Partial endo H digestion analysis. HSV-2-infected cells cultured in the presence of monensin were harvested at 20 h postinfection, solubilized, and subjected to endo H digestion for increasing lengths of time as indicated. Digestion products were analyzed by immunoblotting with anti-pgG-2. Positions of the 104K, 72K, and 31K undigested proteins and the digested 100K, 69K, and 29K products are indicated.

the reactions were terminated by boiling in 1% SDS. The digests were analyzed by immunoblotting with anti-pgG-2 serum (Fig. 5). Upon increasing times of incubation with endo H, both the 72K and the 31K bands were gradually converted into two faster-migrating bands before complete digestion, yielding single 69K and 29K products, respectively. Similarly, faster-migrating partial digestion products that were converted to the 100K component with time were generated from the 104K protein. These results suggest that all four potential N-linked glycosylation sites present on the 104K precursor are glycosylated, with the 72K and the 31K components each containing two of the sites. Furthermore, the cleavage site can be deduced to be located between the glycosylation sites at amino acid positions 163 and 437.

In this study, the orientations of the cleavage products and the location of the cleavage site on the gG-2 precursor were determined with the use of a gG-2-specific monoclonal antibody and antisera directed against two synthetic peptides corresponding to amino acid residues 65 to 78 and 247 to 260 of the predicted sequence of the gG-2 precursor. In support of the antigenicity prediction of Hopp and Woods (13), the synthetic peptides corresponding to amino acids 65 to 78 and 247 to 260 generated two antipeptide sera that reacted with the gG-2 gene products. Both antisera reacted with the 104K precursor (or the unglycosylated 100K protein) and with only one of the cleavage products, the 31K component (or the 34K secreted form). These results map the 31K cleavage product to the amino-terminal portion of the gG-2 precursor which includes the two N-linked glycosylation sites at amino acid residues 104 and 163 and part or all of the region within peptide 2 (residues 247 to 260). Consequently, the monoclonal anti-gG-2 antibody, 13αC6, which contains an epitope mapping within the carboxyl amino acids 486 to 566 of the gG-2 precursor, reacts only with the 72K component and not with the 31K component. These results definitively map the 72K protein to the carboxyl-terminal portion of the gG-2 precursor. The reactivity of these antibodies with only one of the two cleavage products and their common uncleaved precursor confirms the cleavage-processing model for the synthesis of gG-2. The presence of two N-linked glycosylation sites on each of the cleavage products, as suggested by the results of the partial endo H digestion experiment, implies that the cleavage site(s) must fall between the glycosylation sites at amino acid residues 163 and 437. This cleavage site(s) can be narrowed to between amino acid residues 260 and 437 since the 31K product reacts with an antibody directed against residues 247 to 260; therefore, the secreted 34K component is located between amino acid residues 1 and 260 to 437 on the gG-2 precursor.

The amino acid sequence of gG-2 within the 34K secreted component shows limited homology with two well-characterized gG homologs, gX of pseudorabies virus (498 amino acids) (20) and gG of equine herpesvirus 4 (EHV4) (405 amino acids) (7). The major products of both gX and EHV4 gG are also secreted from virus-infected cells (7, 20), although the precise location of cleavage is not understood. Similarity between gX and the 34K protein occurs within a region of 81 amino acids at residues 70 to 150 of gX and 92 to 172 of gG-2 (the 34K protein) with the presence of three cysteines at homologous locations, as well as the occurrence of eight identical residues (17). The EHV4 gG also shares limited sequence similarities with both gG-2 and gX, including the presence of four conserved cysteines and an N-linked glycosylation site within the first 250 amino acids of gG-2, gX, and gG of EHV4 (7). The presence of sequence similarities among the three secreted gG homologs (the 34K protein, gX, and gG of EHV4) implies a shared biological function. The secreted proteins may be involved in facilitating the virus' evasion of the host immune system. Such a potential role for the 34K protein is under study. With the information on the orientation and cleavage region of the gG-2 precursor, specific proteins with mutations in the cleavage region of the gG-2 gene can be constructed to identify the cleavage site and to determine the biological significance of the cleavage event.

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REFERENCES

1. **Ackermann, M., R. Longnecker, B. Roizman, and L. Pereira.** 1986. Identification, properties, and gene location of a novel glycoprotein specified by herpes simplex virus 1. Virology **150:**207–220.
2. **Anderson, D. J., and G. Blobel.** 1983. Immunoprecipitation of proteins from cell-free translations. Methods Enzymol. **96:**111–120.
3. **Aruffo, A., and B. Seed.** 1987. Molecular cloning of a CD28 cDNA by a high-efficiency COS cell expression system. Proc. Natl. Acad. Sci. USA **84:**8573–8577.
4. **Balachandran, N., D. Harnish, W. E. Rawls, and S. Bacchetti.** 1982. Glycoproteins of herpes simplex virus type 2 as defined by monoclonal antibodies. J. Virol. **44:**344–355.
5. **Balachandran, N., and L. M. Hutt-Fletcher.** 1985. Synthesis and processing of glycoprotein gG of herpes simplex virus type 2. J. Virol. **54:**825–832.
6. **Compton, T., and R. J. Courtney.** 1984. Virus-specific glycoproteins associated with the nuclear fraction of herpes simplex virus type 1-infected cells. J. Virol. **49:**594–597.
7. **Crabb, B. S., H. S. Nagesha, and M. J. Studdert.** 1992. Identification of equine herpesvirus 4 glycoprotein G: a type-specific, secreted glycoprotein. Virology **190:**143–154.
8. **Dall'Olio, F., N. Malagolini, G. Campadelli-Fiume, and F. Serafini-Cessi.** 1987. Glycosylation pattern of herpes simplex virus type 2 glycoprotein G from precursor species to the mature form. Arch. Virol. **97:**237–249.

9. **Eberle, R., and R. J. Courtney.** 1980. Preparation and characterization of specific antisera to individual glycoprotein antigens comprising the major glycoprotein region of herpes simplex virus type 1. J. Virol. **35:**902–917.
10. **Frame, M. C., H. S. Marsden, and D. J. McGeoch.** 1986. Novel herpes simplex virus type 1 glycoproteins identified by antiserum against a synthetic oligopeptide from the predicted product of gene US4. Virology **67:**745–751.
11. **Gorman, C.** 1985. High efficiency gene transfer into mammalian cells, p. 143–190. *In* D. M. Glover (ed.), DNA cloning, vol. II. IRL Press, Washington, D.C.
12. **Harlow, E., and D. Lane.** 1988. Antibodies: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. **Hopp, T. P., and K. R. Woods.** 1983. A computer program for predicting protein antigenic determinants. Mol. Immunol. **20:** 483–489.
14. **Hutchinson, L., H. Browne, V. Wargent, N. Davis-Poynter, S. Primorac, K. Goldsmith, A. C. Minson, and D. C. Johnson.** 1992. A novel herpes simplex virus glycoprotein, gL, forms a complex with glycoprotein H (gH) and affects normal folding and surface expression of gH. J. Virol. **66:**2240–2250.
15. **Marsden, H. S., A. Buckmaster, J. W. Palfreyman, R. G. Hope, and A. C. Minson.** 1984. Characterization of the 92,000-dalton glycoprotein induced by herpes simplex virus type 2. J. Virol. **50:**547–554.
16. **McGeoch, D. J., A. Dolan, S. Donald, and F. J. Rixon.** 1985. Sequence determination and genetic content of the short unique region in the genome of herpes simplex virus type 1. J. Mol. Biol. **181:**1–13.
17. **McGeoch, D. J., H. W. M. Moss, D. McNab, and M. C. Frame.** 1987. DNA sequence and genetic content of the *Hin*dIII region in the short unique component of the herpes simplex virus type 2 genome: identification of the gene encoding glycoprotein G, and evolutionary comparisons. J. Gen. Virol. **68:**19–38.
18. **Olofsson, S., M. Lundstrom, H. Marsden, S. Jeansson, and A. Vahlne.** 1986. Characterization of a herpes simplex virus type 2-specified glycoprotein with affinity for *N*-acetylgalactosamine-specific lectins and its identification as g92K or gG. J. Gen. Virol. **67:**737–744.
19. **Powell, K. L., and R. J. Courtney.** 1975. Polypeptides synthesized in herpes simplex virus type 2 infected cells. Virology **66:**217–228.
20. **Rea, T. J., J. G. Timmins, G. W. Long, and L. E. Post.** 1985. Mapping and sequence of the gene for the pseudorabies virus glycoprotein which accumulates in the medium of infected cells. J. Virol. **54:**21–29.
21. **Richman, D. D., A. Buckmaster, S. Bell, C. Hodgman, and A. C. Minson.** 1986. Identification of a new glycoprotein of herpes simplex virus type 1 and genetic mapping of the gene that codes for it. J. Virol. **57:**647–655.
22. **Roizman, B., B. Norrild, C. Chen, and L. Pereira.** 1984. Identification and preliminary mapping with monoclonal antibodies of a herpes simplex virus type 2 glycoprotein lacking a known type 1 counterpart. Virology **133:**242–247.
23. **Roizman, B., and A. E. Sears.** 1990. Herpes simplex viruses and their replication, p. 1795–1841. *In* B. N. Fields, D. M. Knipe, et al. (ed.), Virology, 2nd ed. Raven Press, Ltd., New York.
24. **Su, H. K., and R. J. Courtney.** 1988. Inducible expression of herpes simplex virus type 2 glycoprotein gene gG-2 in a mammalian cell line. J. Virol. **62:**3668–3674.
25. **Su, H. K., R. Eberle, and R. J. Courtney.** 1987. Processing of the herpes simplex virus type 2 glycoprotein gG-2 results in secretion of a 34,000-M_r cleavage product. J. Virol. **61:**1735–1737.
26. **Takasuki, A., and G. Tamura.** 1971. Effect of tunicamycin on the synthesis of macromolecules in culture of chick embryo fibroblasts infected with Newcastle disease virus. J. Antibiot. **24:**785–794.
27. **Tartakoff, A.** 1983. Perturbation of vesicular traffic with the carboxylic ionophore monensin. Cell **32:**1026–1028.
28. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76:**4350–4354.
29. **Weldon, S. K., H. K. Su, J. D. Fetherston, and R. J. Courtney.** 1990. In vitro synthesis and processing of herpes simplex virus type 2 gG-2, using cell-free transcription and translation systems. J. Virol. **64:**1357–1359.
30. **Wenske, E. A., M. W. Bratton, and R. J. Courtney.** 1982. Endo-β-*N*-acetylglucosaminidase H sensitivity of precursors to herpes simplex virus type 1 glycoproteins gB and gC. J. Virol. **44:**241–248.